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Review

Tube or Not Tube: Remodeling Epithelial Tissues by Branching Morphogenesis

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Branching morphogenesis involves the restructuring of epithelial tissues into complex and organized ramified tubular networks. Early rounds of branching are controlled genetically in a hardwired fashion in many organs, whereas later, branching is stochastic, responding to environmental cues. We discuss this sequential process from formation of an organ anlage and invagination of the epithelium to branch initiation and outgrowth in several model systems including *Drosophila* trachea and mammalian lung, mammary gland, and kidney.

As documented by numerous artists, scientists, and writers, the astonishing beauty of structures generated by multicellular organisms has always fascinated human beings. Within the plant kingdom, highly organized patterns can be observed in the branches of trees and the veins of leaves; equally fascinating but less visible are the highly organized spacing patterns in the internal organs of animals such as the mammalian lung, kidney, and mammary gland, or the insect tracheal system (Figure 1). The formation of these ramified organs in animals, a process generally referred to as "branching morphogenesis," involves the restructuring of epithelial tissues to complex but highly organized tubular networks that transport gases and/or produce fluids. Somewhat as a surprise, it turns out that a significant portion of the branching pattern in many of these organs is controlled genetically in a hardwired fashion, giving rise to successive rounds of branching in a predictable manner.

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Secreted factors controlling the branching pattern have been isolated in invertebrates and vertebrates, and developmental themes common to the formation of different branched structures are becoming apparent at the molecular and cellular level. Because epithelial cells have intrinsic architectural features, exhibiting apical and basolateral plasma membrane domains of unique composition and well-defined cell-cell junction complexes, one must assume that branching morphogenesis relies in part on the interaction of the program specifying these features with extracellular factors. We discuss recent findings from studies in insects and vertebrates supporting this view, with special emphasis on the branch-driving processes and the underlying epithelial cellular responses.

The formation of an epithelial organ via branching morphogenesis can generally be subdivided into a series of sequential steps (Figure 2): (1) formation of an organ anlage, often in the form of a placode; (2) invagination of the placode or primary bud formation; (3) branch initiation; (4) branch outgrowth; (5) reiteration of the branching process; and (6) differentiation of organ-specific proximal and distal structures. Because the correct execution of each individual step depends on the proper execution of the previous one, we start from the definition of the organ anlage in different branched organs (*Drosophila* trachea, mammalian lung, mammary gland, or kidney), highlighting salient features of each step, the molecular components involved, and possible common schemes. The mechanisms regulating tubulogenesis, including in the vascular system, are the subject of a recent review in *Cell* (Lubarsky and Krasnow, 2003).

Step 1: Formation of the Anlagen of Branched Organs

During development, cells become determined to their terminal state in a stepwise manner. Organ anlagen are generally marked and determined by the expression of a specific combination of transcription factors and signaling mediators in a group or field of cells, initiating events that determine the further developmental potential of the cellular descendents. Because branching morphogenesis in most cases remodels preexisting epithelia, the anlagen are defined during development by the restricted expression of particular transcription factors in a subdomain of an epithelium that either forms a sheet (fly trachea), or is part of a previously formed tube (vertebrate kidney and lung). In the tracheal system, a combination of at least two regionally transcribed transcription factors, Trachealess (Trh) and Drifter/Ventral veinless (Dfr/Vvl), are important for selecting groups of cells from within a large epithelial sheet to form the tracheal placode and for the subsequent branching morphogenesis process (Wilk et al., 1996; Isaac and Andrew, 1996; Anderson et al., 1995; de Celis et al., 1995). Although not known in detail, it is presumed that the restricted expression of *trh* and *dfr/vvl* in the tracheal anlagen results from the interpretation of previously

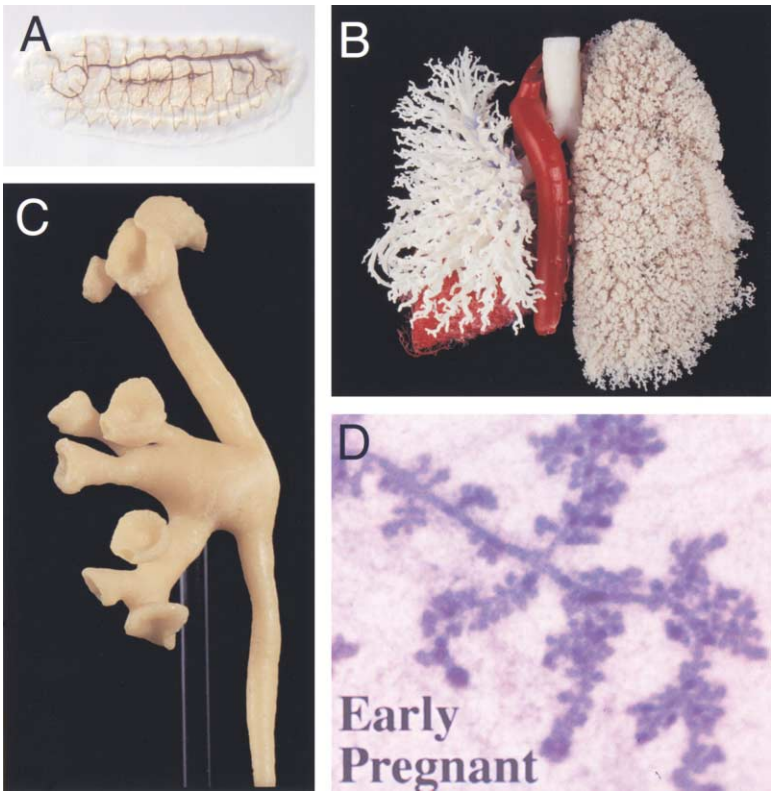

Figure 1. Branching Morphogenesis at the Organ Level

(A) The tracheal system of a stage 15 embryo, as visualized with a luminal antibody, 2A12.

(B) In white, preparation of the lung of an adult human using acryl polyester to fill in the airways. View from behind. The left lung has been filled less than the right half. Courtesy of H. Kurz, Anatomical Museum, University of Basel, Switzerland. In red, the descending aorta is visible.

(C) Collecting ducts of an adult kidney derived from the branched ureteric bud, filled with colored polyester. Courtesy of H. Kurz, Anatomical Museum, University of Basel, Switzerland.

(D) Branching in the mammary gland of a mouse in early pregnancy.

specified anterior-posterior (AP) and dorsoventral (DV) cues by transcriptional control elements.

A prominent role in the initiation of branching is attributed to Trh, as in its absence, all aspects of morphogenesis fail to initiate and the epithelial sheet remains intact. Trh protein is present throughout tracheal development, and it is likely that most or all subsequent decisions taken by tracheal cells are orchestrated in a "tube branching" fashion by Trh in collaboration with its ubiquitously expressed partner Tango (Zelzer et al., 1997; Ohshiro and Saigo, 1997; Sonnenfeld et al., 1997). The Trh/Tango protein complex appears to play the role of a

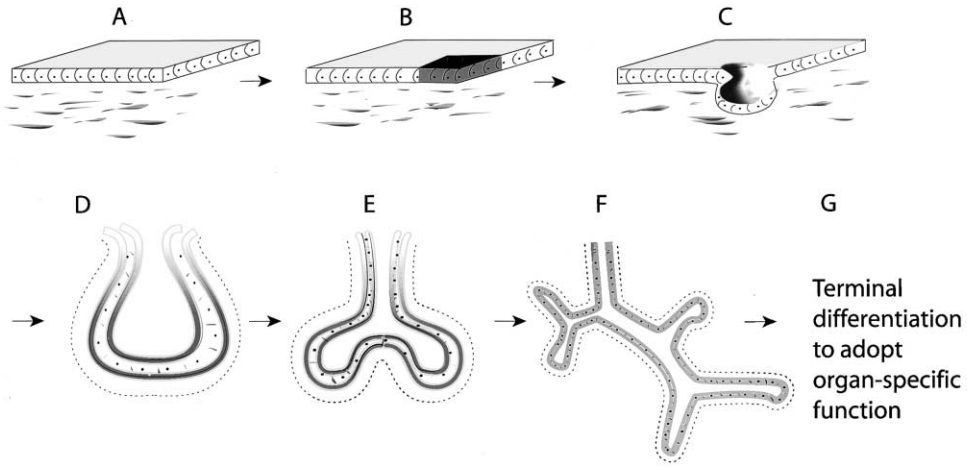

Figure 2. Branching Morphogenesis at the Cellular Level

Schematic representation of a typical branching process. In many cases, a subgroup of cells (schematically illustrated in black in [B]) of a preexisting epithelium (A) is assigned to undergo branching morphogenesis by the expression of a specific subset of transcription factors and/or signaling mediators. As a consequence of this determination step, these cells invaginate or form a primary bud (C). Branch formation is then initiated in the invaginated (or budded) structure (from [D] to [E]) and the branching process can be reiterated numerous times (F). In addition, lateral branches can be induced. After the branching process, complex processes lead to the development of specialized terminal structures, a process that is different in different branched organs. Because the development of the vascular system does not in general follow the scheme outlined in this figure, we have excluded in this review a description of how the branched aspects of the arterial and venous network arises.

tracheal selector complex in the trunk of the developing embryo and activates the transcription of a number of genes encoding molecules essential for the subsequent branching process. It recently became apparent that cell-specific signal responses are generated with the direct participation of selector gene products (Affolter and Mann, 2001; Guss et al., 2001; Curtiss et al., 2002; Mann and Carroll, 2002); indeed, a Dpp/BMP-responsive tracheal enhancer requires the direct binding of the Trh protein in order to be signal activated in a tissue-specific manner (M.A. and R. Schuh, unpublished data). It is very likely that other trachea-specific nuclear responses to various signals also require the participation of Trh/Tango. For Trh to participate in tracheal-specific signal interpretation, the protein has to be present throughout the morphogenesis process, and this appears to be achieved through direct autoregulation. Knowledge of the precise nature of the placode determinant(s) is crucial for a molecular understanding of most events that follow placode determination, when widely distributed signals that play roles in numerous developmental situations have to be interpreted in an "organ-specific" branching context.

In mammals, only one transcription factor has so far been implicated in a role analogous to that of Trh. The labyrinth, a branched epithelium that arises from the chorionic plate that makes the fetal interface with maternal blood in the placenta, results from simple branch formation by evagination of the chorionic trophoblasts. This process is regulated by the *glial cells missing* (*Gcm-1*) transcription factor (Anson-Cartwright et al., 2000). *Gcm1* is expressed in clusters of trophoblast cells within the chorion that will form the invading tips of simple branches. These branches then elongate, bifurcate, and initiate syncytiotrophoblast differentiation.

Interestingly, the anlagen of the mammary gland might arise through cell migration, uniting nonepithelial cells in defined regions (Veltmaat et al., 2003). To better understand these branching processes in vertebrates, it will be important to identify possible organ selector genes and study their participation in the instruction and readout of all subsequent steps.

Step 2: Invagination of the Placode, Primary Bud Formation

The first morphologically visible sign of branching morphogenesis is the invagination of a placode or the evagination of a primary bud, processes that lead to the segregation of those cells that will form the branched organ system away from the surface of a preexisting epithelial structure. The invagination process often generates the initial lumen, which is then expanded by branch formation; in other cases, as in the mammary gland, the lumen is generated secondarily and involves apoptosis (see Hogan and Kolodziej, 2002; Lubarsky and Krasnow, 2003, for excellent reviews on tubulogenesis). Simple invagination/evagination processes are generally thought to arise, at least in part, through apical constrictions, but very little is known at present about the exact events leading to invaginations that subsequently give rise to branched epithelial organs. Epidermal growth factor receptor (EGFR) signaling appears to be required to some extent for the invagination of the tracheal placodes in *Drosophila*, but its cellular targets are unknown (Llimargas and Casanova, 1999). Even the most carefully studied case of an invagination process in the fly embryo, the invagination of the mesoderm, has been rather refractory to detailed genetic analysis; only very few components, including two transcription factors, a GTP exchange factor, a secreted molecule of unknown function, and a cell cycle regulator have been clearly associated with the process thus far. Considering these difficulties in the fly, it will be even more difficult to decipher in individual cases of branching morphogenesis how the invagination process is controlled molecularly.

An important aspect of branching morphogenesis regarding the invagination process is that the tissue remains epithelial, and does not undergo a subsequent epithelial-mesenchymal transition (EMT). During gastrulation of the fly, mesodermal cells undergo a complete EMT after invagination and disperse toward the dorsal side of the embryo. Thus, although mesoderm invagination leads to the formation of a transient lumen, this lumen is not expanded but rather the epithelial sheet disintegrates and no branching process is, or can be, initiated. Clearly, organs undergoing branching morphogenesis during their development have to maintain (or ultimately regain) their epithelial character (O'Brien et al., 2002).

Interestingly, however, the branching process itself might actually rely on and be driven by a partial release of this restriction in the cells at the tip of growing branches, as we will outline below.

Step 3: Initiation of the Branching Process

At the cellular level, an intriguing feature of the development of a branched epithelial organ is the initiation of branch formation. Much has been learned in the past few years about the signaling molecules that initiate this process from the outside. Secreted ligands of the FGF and the BDNF family of signaling molecules play a major role in this process. In the *Drosophila* tracheal system as well as in the mouse lung, FGF molecules (Branchless [Bnl] in the trachea and FGF10 in the lung) have been shown to be strictly required for the branching process.

In the developing tracheal system of the *Drosophila* embryo, the gene encoding the FGF ligand Branchless (Bnl) is expressed in a dynamic fashion in clusters of nontracheal cells at positions toward which branches extend (Sutherland et al., 1996). The Bnl signal is transmitted and interpreted in tracheal cells via the FGF receptor Breathless (Klambt et al., 1992), which accumulates in the placode under the direct transcriptional control of Trh. The Bnl/FGF production in specific locations around the placode is under tight genetic control of the AP and DV genes, and thus identical in each embryo and hardwired.

Recent studies at the cellular level have provided evidence that the Bnl signal results in the formation of dynamic filopodial extension from the basal side of those tracheal cells that are closest to the Bnl source. This demonstrates that Bnl/FGF induces tracheal branch formation by regulating cell motility via cytoskeletal rearrangements (Figure 3; Ribeiro et al., 2002; also see Sato and Kornberg, 2002). Thus, the branching process is initiated by selecting a subgroup of cells that are part

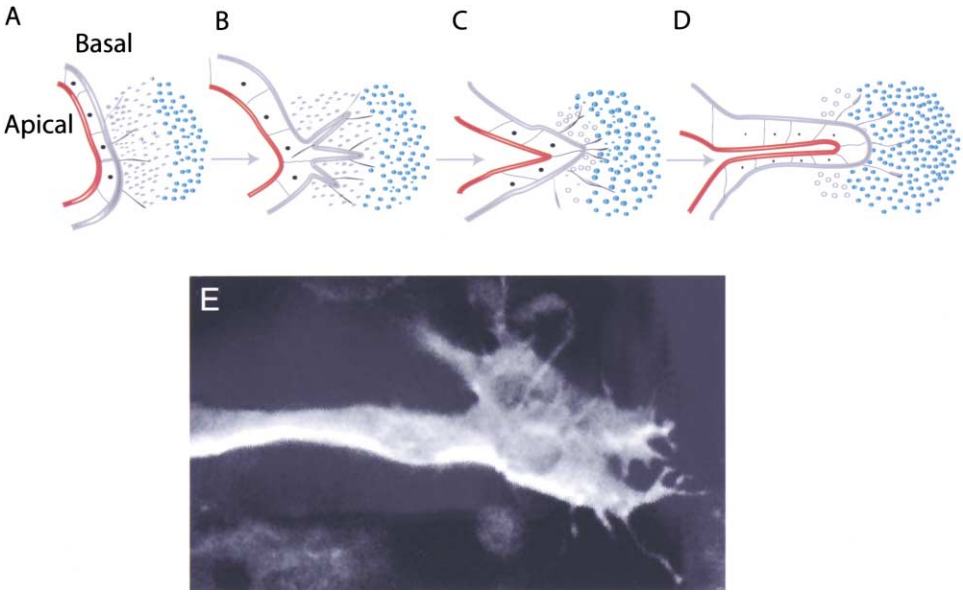

Figure 3. Branching Morphogenesis at the Subcellular Level

Control of branch formation at the subcellular level in the *Drosophila* tracheal system. The FGF receptor tyrosine kinase Breathless is expressed in all tracheal cells. The activation of the receptor in the cells at the tip of the outgrowing branches, presumably due to their proximity to the localized source of the FGF ligand Branchless (blue), leads to the formation of filopodial cell extensions (A). Cells at the tip of the bud subsequently form broader cell extensions (B), and ultimately move toward the Bnl source (C and D). In (E) is shown a confocal image of a tracheal branch of a stage 14 *Drosophila* embryo expressing a membrane-bound version of GFP specifically in tracheal cells. One can clearly see that only the two leading cells produce filopodia (see also Sutherland et al., 1996; Ribeiro et al., 2002).

of a polarized epithelia and inducing them to become motile and invasive, suggesting a partial transformation away from a strict epithelial phenotype as a factor initiating branching. This invasive behavior might be a general feature or even the driving force of branching morphogenesis, because branches enter surrounding tissues. Although the specific effects on cellular behavior are less well understood in the mouse, it is striking to note that FGF10 is dynamically expressed in the splanchnic mesoderm of the lung, and signals through FGFR2b and/or -1b to the lung epithelium in an analogous way. However, in other tissues, other mesenchymal growth factors seem to play this role, such as the kidney, where GDNF and not FGF10 seems to be relevant.

Interesting results regarding this issue were recently obtained in three-dimensional culture of Madin-Darby canine kidney (MDCK) cells (summarized in O'Brien et al., 2002). Single MDCK cells proliferate to form cysts when embedded in a collagen I matrix. MDCK cysts resemble epithelia in vivo; both are polarized monolayers enclosing a lumen and encircled by a basement membrane. When exposed to the mesenchymally derived hepatocyte growth factor (HGF), MDCK cysts grow branching tubules, a response dependent on matrix metalloproteinases (MMPs) that is also stimulated in vivo. It appears that while epithelia possess an intrinsic differentiation program to impose the formation of polarized, lumen-enclosing monolayers, growth factors such as HGF induce a transient, partial dedifferentiation to promote the growth of tubules and secondary branches (Pollack et al., 1998). However, the importance of this activity in vivo is not entirely clear, as mouse embryos null for HGF die of placental failure, with apparently normal kidneys. Experiments done in tissue culture and in vivo indicate that, during mammary gland development, a partial EMT, dependent on stromelysin-1 (MMP-3) from mesenchymally derived cells may occur at the tip of growing branches (Simian et al., 2001; B. Wiseman and Z.W., unpublished observations).

In the mammary gland, factors that initiate and control the outgrowth of individual branches are just being elucidated. Although it is likely that cell migration represents a driving force for branching and the invasion of the epithelial cell layer into the fat pad, branches may be pushed outward by cell division rather than pulled out by migratory cells at the tip. Future studies will address this issue. What is clear at present is that the molecules regulating these processes are different during the formation of primary ducts and secondary branches (Wiseman and Werb, 2002).

Step 4: Branch Outgrowth or Elongation

The tracheal system in hatching *Drosophila* larvae represents a simple branched organ in which the branching pattern is governed by cell shape changes and cell movement in the absence of concomitant cell division (Samakovlis et al., 1996). However, recent studies suggest that even in this simple situation, the chemoattractive forces of Bnl/FGF are not sufficient to produce productive branch outgrowth. Additional programs have to be initiated in subsets of tracheal cells to allow branch morphogenesis, ultimately resulting in branch outgrowth and formation. In the case of the unicellular dorsal branch, for example, Dpp-induced activation of the Knirps/Knirps-related zinc finger transcriptional regulators is essential for productive branch outgrowth toward

Bnl (Vincent et al., 1997; Chen et al., 1998; Ribeiro et al., 2002). Thus, chemoattraction has to be coupled to an appropriate, branch-specific epithelial response that in many cases might include a capacity to undergo cell intercalation, convergent extension, or other cell rearrangements.

Clearly, in all the vertebrate systems that we discuss, branch outgrowth and elongation are associated with cell division, and thus the former must somehow be coupled to the latter. It remains to be determined whether the chemoattractant(s) regulate cell division directly, or whether other molecules (with stimulating and/or inhibitory effects) are controlling cell migration in distal parts of developing branches.

Extensive remodeling of the basement membrane and selective modulation of the cellular adhesion to the ECM occurs during the extension process in the mammalian systems. Molecules involved in regulating branch outgrowth include matrix proteins, matrix-degrading proteinases that are secreted by various cells, as well as cell adhesion molecules expressed on cell surfaces. In addition, programmed cell death might contribute to the morphology of branching organs, particularly in formation of a lumen (Debnath et al., 2002). As very little is known about these aspects of branch outgrowth, we will not discuss them in detail here.

Step 5: Spatial Organization of Successive Branching Events

The most fascinating aspect of branched organs to a nonscientist is their aesthetic appearance (Figure 1). Numerous branches refine into sequential series of finer and finer branches, and despite the occurrence of millions of such branches in the mature lung, for example, order appears to prevail. Understanding the molecular network controlling the sequential establishment of this pattern represents a fascinating challenge.

From comparative studies between different individuals, it appears that the reproducible, dichotomous branching during 16 successive generations in the human lung must be controlled genetically in a relatively hardwired manner (Warburton et al., 2000). In the last few years, tremendous progress has been made in identifying genes in the mouse that are instrumental in inducing distal branching and genes that prohibit branching more proximally. Epithelial-mesenchymal crosstalk at the tip results in modulation of FGF signaling, ensuring dichotomous branching, and refinement of branches at each successive step (Figure 4).

Although many questions remain, molecular scenarios that partially account for the reiterative feature of successive branching steps are emerging. It appears that one of the key aspects to be controlled is the FGF signaling pathway, both by fine-tuning the FGF10 levels in time and space in the distal mesenchyme as well as by modulating the response in the adjacent epithelium along the proximal-distal axis. Key regulators in this process are Shh, BMP-4, TGFβ, and receptor tyrosine kinase signaling modulators of the Sprouty family. A simplified model accounting for their interplay, which ultimately leads to reiterated branching, is emerging (for an excellent review on this topic, see Cardoso, 2001).

As described above, localized FGF10 expression in the distal mesenchyme is responsible for the formation of buds in the lung branching process. While little is known about how islands of mesenchymal cells are determined in a stereotyped fashion to secrete FGF10 and stimulate bud outgrowth, two important regulators expressed at high levels in the distal epithelium, Shh and BMP-4, have been identified. Shh appears to be part of an epithelial network of regulators that restrict FGF10 expression; in *shh*$^{-/-}$ lungs, widespread expression of FGF10 is observed in the mesenchyme, resulting in a failure of epithelial tubules to branch properly. It has been proposed that the growing epithelial bud, which expresses high levels of Shh (independent of FGF10), interacts with the chemotactic source FGF10 as it approaches it. This interaction leads to a reduced expression in the immediate vicinity of the approaching tip and a splitting and lateral displacement of the FGF10 source.

While Shh regulates the spatial distribution of the chemoattractant FGF10, the second regulator, BMP-4, is part of a distal epithelial signaling center that negatively controls proliferation and regulates proximal-distal differentiation (Bellusci et al., 1996; Weaver et al., 1999, 2000). Because increasing FGF10 levels raise BMP-4 levels, this crosstalk serves to limit bud outgrowth during branching. An additional gene that is transcriptionally induced as branches approach the FGF source and that most likely contributes to limit branch outgrowth is *Sprouty2* (*Spry*; Mailleux et al., 2001). *Spry* genes encode a family of cysteine-rich proteins that antagonize receptor tyrosine kinase signaling (Hacohen et al., 1998; Casci et al., 1999). *Spry2* mutant mice have not been analyzed yet for a possible lung phenotype, but ectopic expression experiments indicate that *spry2* is part of a negative feedback loop by which increased FGF signaling in the most distal lung epithelium limits FGF signaling in responding cells, ultimately resulting in decreased cell proliferation. Clearly, many important aspects remain to be addressed; nevertheless, the interplay of these three regulators with FGF10 suggests ways to regulate the approach and stalling of a bud toward the chemoattractant.

How is the dichotomous branching process induced, so that the whole process just described can start all over again and be reiterated many times? In addition to BMP-4 and Spry-2, TGFβ-1 plays an important role in this process (as well as in other processes in lung formation that we will not consider here). TGFβ-1 accumulates at sites of cleft formation and along proximal airways. TGFβ-1 promotes synthesis of extracellular matrix that, when deposited in the epithelial-mesenchymal interface, might prevent local branching (Figure 4). FGF10 now acts mainly on lateral epithelial cells, and helped by a lateral relocalization of the FGF source under the control of Shh, generates two new distal ramifications according to the mechanisms described above (see Figure 4 and legend).

Novel molecules are involved in the control of branching processes by surrounding cells in the mammary gland. The mammary gland develops postnatally by branching morphogenesis, creating an arborized ductal system on which secretory lobuloalveoli develop at pregnancy. Sophisticated epithelial-stromal recombination experiments convincingly demonstrated that the

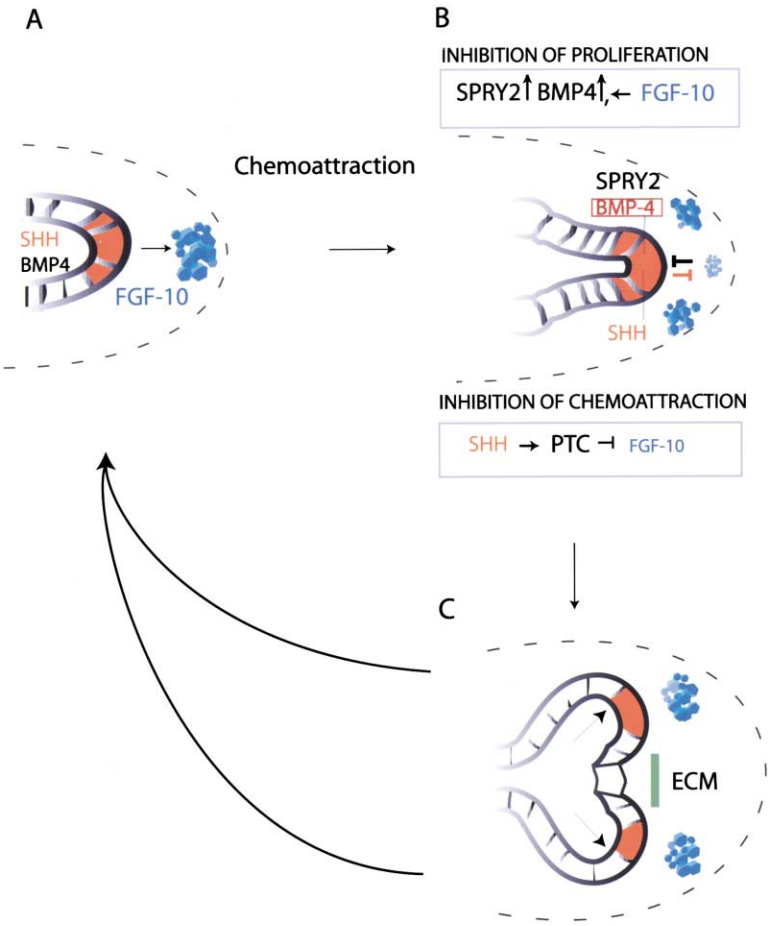

Figure 4. A Molecular Model for Branching Reiteration

Control of reiterating bud formation during lung branching morphogenesis.

(A) Local expression of FGF10 (blue) in the mesenchyme induces chemoattraction and epithelial proliferation. Shh (red) is expressed at the tip of growing branches, while BMP-4 is expressed throughout the epithelium, but at increased levels in the tip cells.

(B) As the bud approaches the chemotactic source of FGF10, Shh inhibits FGF10 expression. In addition, the FGF10-induced increase in BMP-4 levels in the tip cells leads to an inhibition of proliferation.

(C) During distal ramification, FGF10 expression is bifurcated and relocalized laterally, leading to two new sources of the chemoattractant. The processes described in (A) and (B) are reinitiated, and two new distal branches form. At the site of cleft formation, TGFβ induces the synthesis of extracellular matrix components (green bar) that are deposited in the epithelial-mesenchymal interface and prevent local budding.

classical endocrine mammogens estrogen, progesterone, growth hormone, and prolactin, which stimulate the branching process, act on stromal cells (Hovey et al., 2002). However, how this affects stromal influences on the epithelium is not yet clear. At the moment, there is no evidence that chemoattraction plays a role in directing mammary bifurcation and extension, and it remains an open question as to which substances control the tight coupling between proliferation and morphogenesis. What guides the branches? Certainly the interplay of epithelial adhesion receptors with the extracellular matrix (ECM) is essential.

Despite the lack of knowledge on how primary (and lateral) branches are "guided," much has been learned in recent years about the role of proteases in the control of the branching process. Metalloproteinases are both upstream and downstream of EGF receptor signaling, with the ADAMs-type proteases controlling ligand activation and MMPs regulating growth factor function and branching (Wiseman and Werb, 2002; Simian et al., 2001; Kheradmand et al., 2002; Kheradmand and Werb, 2002) in both mammary gland and lung. What is significant is that MMPs are made almost exclusively in the mesenchymal compartment. As such, they are critical mediators of the epithelial-mesenchymal crosstalk and the transient EMT needed for a branch to form. Intriguingly, MMPs may directly regulate migratory activity by cleaving ECM molecules such as laminin-5, turning it into a motogen (Koshikawa et al., 2000). Metalloproteinases and their inhibitors also control epithelial survival (Fata et al., 2001) and thus may regulate lumen formation. At least part of the function of the MMPs is to activate TGFβ, which inhibits lateral branching (B. Wiseman and Z.W., unpublished observation). Whether the MMPs in *Drosophila* also participate in these processes remains to be determined, but initial experiments suggest a role in tracheal remodeling (Page-McCaw et al., 2002).

Step 6a: Branch Size Determination

An emerging issue in branching morphogenesis concerns the control or regulation of tube size. In most systems, the diameter of the tube narrows at successive branching steps but little to nothing is known about the genetic and cellular mechanisms that control tube size. Recent studies in *Drosophila* have started to provide some insight into this issue. The primary branches of the tracheal system have characteristic sizes that are under tight transcriptional control. Branch size depends on interactions between the trachea and the environment, ensuring that properly sized branches invade given tissues. For example, dorsal and ventral branches develop into fine, unicellular tubes under the control of the *knirps/knirps-related* transcription factors (Chen et al., 1998); the transcription of these genes is activated under the control of Trh as a response to the Dpp (BMP2/4) signal, which itself is produced in dorsal and

ventral ectodermal cells (Vincent et al., 1997). In contrast, the dorsal trunk develops into a multicellular large tube under the control of the *spalt/spalt-related* genes (Kuhnlein and Schuh, 1996), itself under the control of Wnt signaling from the cellular environment (Llimargas, 2000; Chihara and Hayashi, 2000). Interestingly, cellular analysis and manipulation of tracheal cell number demonstrate that the size of a tube is not dictated by the specific number or shape of the tracheal cells that constitute it. Rather, tube size appears to be controlled by coordinately regulating the apical (luminal) surface of tracheal cells (Beitel and Krasnow, 2000). How *knirps/knirps-related* and *spalt/spalt-related* genes control the apical surface remains a mystery. Although it is likely that tube caliber is also under transcriptional control in the vertebrate systems discussed here, virtually nothing is known about the molecules involved and the cellular processes regulated. A first clue comes from studies showing that mammary duct caliber may be controlled by myoepithelial cells and epimorphin, which act to control the transcriptional regulator C/EBP (Hirai et al., 2001). Future studies will have to address these issues.

Step 6b: Organ-Specific Terminal Differentiation

The determination of specialized cell types at the periphery of a branched organ is by itself a most fascinating process. These cells form the boundary and the interaction surfaces between the branched organ and the adjacent tissues. Whether the subject is the alveoli in the lung, the glomeruli in the kidney, the lobuloalveoli in the mammary gland, or even the terminal cells of insect trachea, these terminal units provide the cellular machines to carry out specialized functions, often over many years or decades. One of the purposes of the branching process is to increase a hundred- to a million-fold the number of these terminal structures. In the lung, the complex branching pattern of the airways also ensures that during postnatal life, air is properly cleared of particulates, humidified, and distributed evenly to all alveolar units. Clearly, issues regarding the terminal differentiation of branched organs cannot be appropriately discussed in the context of our review, and we refer the reader to excellent recent reviews on these issues (Cardoso, 2001; Hovey et al., 2002; Warburton et al., 2000; Zelzer and Shilo, 2000).

Cell Renewal in Branching Organs?

At first glance, the mammary gland represents a specific challenge in that stem cells capable of repeated cycles of growth are embedded in the ductwork (Welm et al., 2002). But a similar phenomenon appears to occur in *Drosophila*, where tracheoblasts repopulate the tracheal system after each larval state. Interestingly, recent elegant work has shown that during the formation of the adult tracheal system in the pupal period, FGF functions as a mitogen for tracheal cells, and possibly acts on differentiated tracheal cells to induce mitotic and migratory functions (Sato and Kornberg, 2002). It is too early to draw any parallels between the different systems with regard to tissue renewal and stem cells, but without doubt, this line of research will be a major focus in the near future.

Summary and Perspective

Much information has become available in the past few years regarding the genetic control underlying branching morphogenesis. In addition, cellular events involved in the process are now under investigation in several systems. Although we can certainly not appreciate the complexity of the process yet, common scenarios are starting to emerge. It comes as a surprise that organs without evolutionary relatedness, the insect tracheal system and the vertebrate lung, use the same signaling pathway (the FGF pathway) to drive the branching process. One model for the mechanism by which this commonly deployed pathway promotes branching is that chemoattractants may induce a partial transformation of cells away from a strict epithelial phenotype toward a motile and invasive phenotype. Little is known about the molecular components that link the chemoattractant receptors to the cytoskeleton, but the selector proteins present in the responding cells of the branching organ prime the latter for a chemotactic response. The driving force behind the tubular aspect of the branched organs is their epithelial nature, and branch outgrowth needs a tight correlation between cell movement, cell division, and cell shape changes. Molecular networks are emerging, and their effects at the cellular level start to provide a more coherent view of the branching process. It seems likely that many of the cellular events underlying the branching process will prove similar in different organ systems, and that branching regulators target similar subcellular events.

Acknowledgments

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